2006

Modulating lipolysis for nutraceutical and cosmeceutical applications

Mary Katherine Caruso
Louisiana State University and Agricultural and Mechanical College, mcarus1@lsu.edu

MODULATING LIPOLYSIS FOR NUTRACEUTICAL AND COSMECEUTICAL APPLICATIONS

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Human Ecology

by
Mary Katherine Caruso
B.S., Louisiana State University, 2003
May 2006

DEDICATION

This thesis is dedicated to my grandparents, Lil and Roy Tidwell, who both lost their lives on August 29, 2005, due to the horrific effects of Hurricane Katrina. I know that you are both extremely proud of my accomplishments. You are my two angels, and I know you are both looking down on me and watching over me.

ACKNOWLEDGEMENTS

I would like to thank Dr. Frank Greenway for your guidance and diligence during my time as a graduate student as well as an undergraduate student. Thank you for the opportunities and experience that you have given me. You have challenged me to rise above my own expectations, and I sincerely respect and admire you. I truly enjoy working with you.

I would also like to thank my other committee members and instructors, Dr. Maren Hegsted, Dr. Michael Keenan, and Dr. Roy Martin. You have been extremely helpful and encouraging during my time as a graduate student, and I truly thank you for your guidance and support.

I want to thank everyone who played a vital role in my graduate research: Andrew Roberts, Dr. Thomas Guillot, Ying Yu, Mary Beth Burnett, and Annette Hutchison. Your thoughtfulness and help has sincerely been appreciated.

I would like to give a very special thanks to the Bissoon Institute of Mesotherapy for funding my research. Leslie and Dr. Bissoon, thanks for having me at the seminars.

Most of all, I would like to thank my family: Mom, Dad, Annie, and Tony, who have given me unconditional love and support throughout the years. Mom and Dad, I know this year has been extremely difficult for both of you, yet you have faith and courage that you will get through everything. I deeply admire and respect you for the sacrifices you have made and for the life lessons you have taught me. You truly are my heroes!

Lastly, I would like to especially thank my fiancé Eric for his motivation and encouragement. Thank you for always being there during those stressful and difficult times. I love you dearly!

TABLE OF CONTENTS

DEDICATION……..... ii

ACKNOWLEDGEMENTS…… iii

LIST OF TABLES…….. vi

LIST OF FIGURES…… vii

ABSTRACT…… viii

CHAPTER

1 INTRODUCTION……. 1
- Obesity…… 1
- Obesity Intervention Mechanisms and the Role of Lipolysis……. 2
- Nutritional Treatments for Obesity……. 4
- Prescription Treatments for Obesity and Dietary Herbal Supplements……... 5
- Cosmetic Treatments for Obesity ...…… 8
  - Fat Reduction…….. 8
  - Fat Redistribution……..…. 9
  - Mesotherapy……... 10

2 TOPICAL FAT REDUCTION FROM THE WAIST……... 12
- Background……..... 12
- Methods…….. 13
- Results…… 14
- Discussion…….. 16

3 THE COST-EFFECTIVENESS OF THREE DIFFERENT MEASURES OF BREAST VOLUME…….. 18
- Introduction…… 18
- Methods…….. 19
- Analysis……. 21
- Results…… 21
- Discussion…….. 23

4 AN EVALUATION OF MESOTHERAPY SOLUTIONS FOR INDUCING LIPOLYSIS AND TREATING CELLULITE…….. 27
- Background……..... 27
- Methods…….. 27
- Results……..... 28
- Discussion…….. 34

5 SUMMARY AND CONCLUSIONS……..... 37

REFERENCES…….. 40

APPENDIX A: COPYRIGHT PERMISSION FOR PUBLISHED ARTICLE……..……... 44

APPENDIX B: COPYRIGHT PERMISSION FOR ACCEPTED ARTICLE……. 45

VITA…….. 46

LIST OF TABLES

Table

2.1 Changes in waist circumference (cm) from baseline in the two groups and separately for men and women……. 15

2.2 Changes in waist to hip ratio (cm) from baseline in the two groups and separately for men and women…… 16

3.1 Measurements of cup sizes AA, A, B, C, and D…..……... 22

3.2 Relative number of subjects and cost to detect a 5% change in breast volume……. 23

4.1 Stimulation of lipolysis by compounds used in mesotherapy……. 33

LIST OF FIGURES

Figure

1.1 Lipolysis in human fat cells……... 4

4.1 Lipolysis fold inductions of isoproterenol, aminophylline, yohimbine, compared to assay buffer……..… 29

4.2 Lipolysis fold inductions of melilotus alone and in combination with aminophylline compared to assay buffer……..… 30

4.3 Lipolysis fold inductions of isoproterenol alone and in combination with aminophylline compared to assay buffer……..… 31

4.4 Lipolysis fold inductions of isoproterenol and aminophylline in combination, with and without lidocaine compared to assay buffer …….. 32

4.5 Lipolysis fold inductions of isoproterenol, aminophylline, and yohimbine in combination, with and without lidocaine compared to assay buffer …….. 33

ABSTRACT

The first study was conducted to determine if aminophylline cream application to the waist will reduce waist circumference compared to a control. Topical fat reduction from the thigh in women has been demonstrated, but local fat reduction in other areas or in men has not. Fifty men and women were randomized to 0.5% aminophylline cream to the waist twice a day or to no treatment to the waist. At week 12 there was a significant reduction in BMI from baseline that was not different between the groups. The reduction in waist circumference was 11 $\pm$ 1.0 cm in the aminophylline cream group and 5.0 $\pm$ 0.6 cm in the control group ($p<0.001$). The reduction in waist circumference was significant for both sexes, but women lost significantly more waist girth. The waist-to-hip ratio declined, aminophylline levels were undetectable, and there were no adverse events.

The second study developed a cost-effective method of breast measurement that will allow our research team to test the concept of fat redistribution. Breast volume measurements were compared using the Grossman-Roudner cone, plaster casting, and MRI. Five women with breast sizes AA, A, B, C, and D had three volume measures repeated three times. For a single volume measurement, the costs were: $1 for the Grossman-Roudner cone, $20 for the cast, and $1,400 for the MRI. The relative cost for volume measurements using the cast was 64-189 times greater, and using the MRI was 373-33,500 greater than the cost of the Grossman-Roudner cone.

The final study used a human fat cell assay to determine the capacity of currently used mesotherapy solutions to stimulate lipolysis and to determine the effect of combining a local anesthetic to the solutions. The fold induction of the mesotherapy solutions measured by glycerol generation was used to determine their capacity to stimulate lipolysis. Isoproterenol ($p<0.002$), aminophylline ($p<0.00004$), and yohimbine ($p<0.001$) stimulated lipolysis compared to the buffer. The lipolysis stimulated by melilotus and isoproterenol was enhanced by

aminophylline ($p<0.001$). Lidocaine inhibited lipolysis when added to aminophylline and isoproterenol (NS compared to buffer), and when added to aminophylline, isoproterenol, and yohimbine ($p<0.05$ compared to control).

CHAPTER 1

INTRODUCTION

Obesity

Obesity is defined by a body mass index (BMI) greater than 30 kg/m^2, overweight as a BMI between 25 and 30 kg/m^2, and morbid obesity as a BMI greater than 40 kg/m^2 or greater than 35 kg/m^2 in association with obesity-related disease expected to improve with weight loss. Approximately 66% of the United States population is overweight and over 30% are obese. Approximately 5% have a BMI greater than 40 kg/m^2 (Hedley et al., 2004 and Freedman et al., 2002). Flegal describes obesity as an "epidemic" which gives the implication that obesity is a characteristic of entire populations, not just of individuals (1999).

Obesity is defined as a BMI above 30 kg/m^2, because, at that level the risk of death is 1.25 to1.5 times higher than lean controls, and the definition of diastolic hypertension above 90mmHg or total cholesterol above 200 mg/dL is defined by similar increases in risk (Bray, 1996). Obesity increases the relative risk of diabetes, hypertension, dyslipidemia, gallbladder disease, and sleep apnea by more than three fold (WHO, 1997). Morbid obesity, or class III obesity, has been named so because of the high incidence of serious medical diseases associated with it. In the morbidly obese, the prevalence of elevated triglycerides and low HDL cholesterol is 40%, of diabetes or impaired glucose tolerance is 34%, of hypertension is 26%, and of unemployment is 62% (Greenway, 1996). Studies have shown that for people with a BMI greater than 40 kg/m^2, the increase in the relative risk of death was 2.58 times higher in men and 2 times higher in women in comparison with people whose BMI was between 23.5 and 24.9 kg/m^2 (Stevens et al., 1998). Research has concluded that between 112,000 and 325,000 deaths each year can be accredited to obesity (Allison et al., 1999 and Flegal et al., 2005).

Not only is obesity a detriment to one's health, but also a detriment to the economy. Wolf and Colditz describe the estimated cost of obesity, which is attributed to associated diseases such as type 2 diabetes mellitus, coronary heart disease, hypertension, gallbladder disease, osteoarthritis, and breast, endometrial, and colon cancer (1998). The National Health Interview Survey was used to estimate that obesity cost the United States approximately $100 billion dollars in 1995.

In addition to the numerous health and economic issues of obesity, there is a very powerful stigma associated with both overweight and obesity. A discrimination and prejudice has become common towards the overweight and obese (Puhl and Brownell, 2001). In a study performed by Crandall, parents discriminate against their own overweight children (1995). Thin siblings received greater financial support from their parents than their own overweight siblings, regardless of income, education, or family size. In a study performed by Brylinsky and Moore, children as young as three years old referred to overweight children as ugly, stupid, and mean (1994). Another study by Latner and Stunkard produced astonishing results (2003). Children would rather have a friend with a handicap, such as being in a wheelchair, having an amputated hand, or having a facial deformity rather than having an obese friend.

Obesity Intervention Mechanisms and the Role of Lipolysis

Obesity is caused by an energy imbalance. People who are overweight or obese eat more calories than they expend. Fat is a dynamic storage depot of body energy. Calories from the food people eat are stored in the fat during the day and some of that fat is mobilized at night to meet energy needs until breakfast the next day through lipolysis. Thus, lipolysis is one of the mechanisms that has promise for intervention in this energy imbalance problem.

Angiogenesis is another mechanism that has the potential to treat energy imbalance. Inhibitors of angiogenesis reverse obesity in rodent models, and an assay based on human fat

tissue has been validated for use in screening angiogenesis inhibitors for the treatment of obesity (Roberts et al., submitted, 2005). Using this assay, gallic acid was discovered to be a plant ingredient with angiogenic inhibitory properties. A clinical trial of gallic acid for the treatment of obesity was unsuccessful and subsequent studies demonstrated that gallic acid is not well absorbed from the gastrointestinal tract (Roberts et al., submitted, 2005).

Although there are many other examples of mechanisms by which one might intervene to treat obesity, this thesis focuses on lipolysis as a mechanism by which adipose tissue can be regulated. Lipolysis is defined as the hydrolysis of lipids, or simply the process of dissolving fat. This happens when one diets and forces his or her body to burn stored fat. During this process hormone sensitive lipase breaks down fat into glycerol and free fatty acids, which are released into the bloodstream, where they may be either re-esterified by adipocytes or metabolized (Large et al., 2004). Thus, lipolysis plays a central role in the regulation of fat mass composed of human fat cells, or adipocytes. There are two main pathways involved in lipolysis: the beta-adrenergic pathway, which is cyclic AMP mediated, and the natriuretic peptide pathway, which is mediated by cyclic GMP (Robidoux et al., 2004). Recent evidence suggests that the novel natriuretic peptide system promotes lipolysis only in primate adipocytes.

The classic pathway for lipolysis in fat cells starts with activation of the beta-adrenergic receptor by beta-agonists via the G_s proteins in adipocytes (Robidoux et al., 2004). This leads to the activation of adenylate cyclase, which then increases cyclic AMP levels. Elevated cyclic AMP behaves as a second messenger, which activates hormone sensitive lipase. Hormone sensitive lipase, the rate-limiting enzyme regulating adipocyte lipolysis, then catalyzes the hydrolysis of triglycerides and results in the release of glycerol and free fatty acids. This process causes increased lipolysis. Enzymes called phosphodiesterases hydrolyze cyclic AMP into 5' AMP, which causes a decrease in lipolysis. Through a series of reactions, catecholamines, such

as epinephrine and norepinephrine, will either stimulate lipolysis via β-adrenergic receptors or inhibit lipolysis via α-adrenergic receptors or adenosine receptors in fat cells.

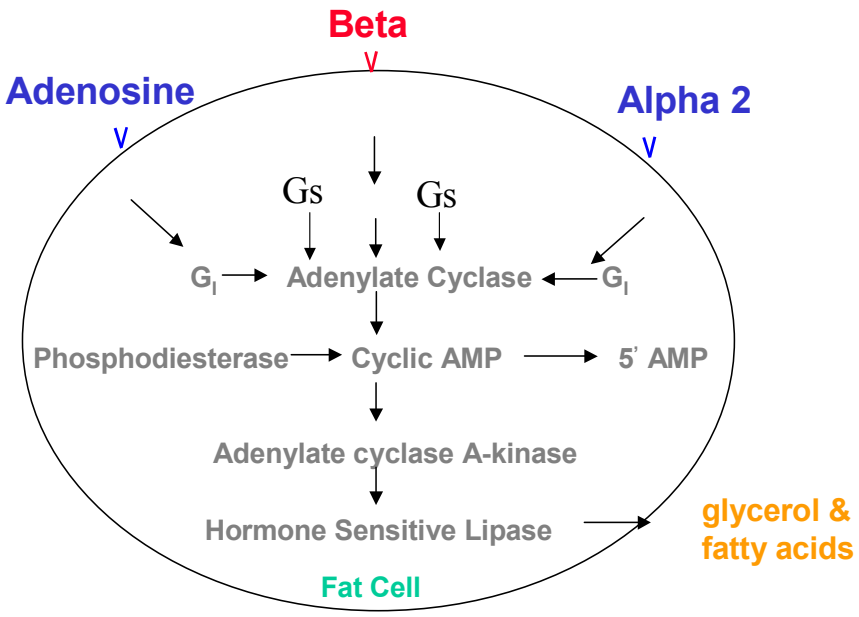

Figure 1.1. Lipolysis in Human Fat Cells

Nutritional Treatments for Obesity

Since obesity is a result of eating more energy than one expends, restricting calories through dieting has been the mainstay of obesity treatment along with behavior changes that reduce calorie intake and encourage activity-associated energy expenditure. Since obesity increases the risk for cardiovascular disease by two to three-fold, interest has also focused upon food choices that will further reduce this risk outside the mere restriction of calories. One example of this approach will be described in some detail. A chocolate covered snack bar

containing 14 grams of full fat rice bran, 6.25 milligrams thiamine, 125 milligrams ascorbic acid, 6.25 milligrams pyridoxine, 37.5 micrograms cyanocobalamin, 50 units tocopherol, 0.125 milligrams folic acid, 500 milligrams of omega-3 fatty acids and 220 calories was developed and compared to a placebo bar without vitamins, similar calories and corn starch/corn oil instead of rice bran/omega-3 fatty acids (Caruso et al., 2005.). This bar was tested to determine its effects on total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, C-reactive protein, and homocysteine. Nine of the ten healthy subjects were on no regular medications, the men were 25-45 years old, the women were 25-55 years old, LDL was between 130-190 mg/dl, and triglycerides were between 150-300 mg/dl. This trial was completed using a randomized double-blind crossover design with balanced order treatments. Subjects incorporated six bars/day in a weight maintaining diet. Homocysteine and lipids were drawn twice at the end of each four-week treatment period and compared by paired t-test. Homocysteine decreased more during the rice bran bar period than during the placebo period (7.1 vs. 7.8 mcmol/L, p=0.028). Total cholesterol, LDL, triglycerides and C-reactive protein decreased more and HDL increased more during the rice bran bar period, but the changes were not statistically significant. This bar for cardiovascular health decreased serum homocysteine, a recognized cardiovascular risk factor. Although 6 bars per day may not be a practical method of reducing cardiovascular risk, the study demonstrates that using foods that function to improve health is a viable approach with scientific support.

Prescription Treatments for Obesity and Dietary Herbal Supplements

The development of drugs for the treatment of obesity began at the end of the nineteenth century with the discovery of thyroid hormone (Putnam, 1893). Over the past one hundred years, obesity drugs have had a disastrous safety record. Amphetamines were associated with drug abuse, dinitrophenol was associated with death from hyperthermia, and most recently

fenfluramine was withdrawn from the market due to an association with cardiac valvular insufficiency (Greenway and Caruso, 2005). In part due to this dismal safety record, the medical profession has looked to drugs approved for purposes other than obesity that have demonstrated an acceptable record of safety in chronic therapy. Bupropion, a drug approved for the long-term treatment of depression has been demonstrated to give weight loss (Anderson et al., 2002). Looking to drugs that stimulate lipolysis is another mechanistic approach that also deserves scrutiny. One drug that fits into that category is the lipolytic stimulator, brain natriuretic peptide, which is an approved treatment for congestive heart failure. It is an expensive protein that is not orally active, both being obvious drawbacks for use as an obesity treatment.

Dietary herbal supplements, which are technically classified as food, are another potential source of lipolytic stimulators. Ephedra was a dietary herbal supplement that stimulated the beta-adrenergic receptor and caused lipolysis, but it was removed from the market for cardiovascular toxicity. Ephedrine combined with caffeine has been used to treat asthma since the 1930's, and it was an approved and successful weight loss drug in Denmark for over a decade.

In a clinical trial performed in Denmark for approval as an obesity drug, 180 obese subjects were randomized to placebo, caffeine 200 mg, ephedrine 20 mg, or caffeine and ephedrine three times a day for six months. The groups on caffeine or ephedrine lost no more weight than placebo and did not differ from each other, but the group on caffeine with ephedrine lost significantly more weight than placebo. Another study by the same group illustrated that caffeine and ephedrine increase resting energy expenditure as part of their mechanism in causing weight loss. In another study performed by Vukovich et al., 20 mg of ephedra combined with 150 mg of caffeine resulted in an increase in resting energy expenditure, heart rate, and blood

pressure (2005), but other studies have shown that blood pressure and heart rate return to placebo levels by eight weeks (Astrup et al., 1992).

Despite its many beneficial effects, ephedra has recently been taken off of the market due to its negative cardiovascular effects. In a study performed by Haller and Benowitz, 140 reports of adverse events were reviewed and were considered related to the use of dietary supplements containing ephedra alkaloids. These 140 reports were selected from all those submitted to the FDA between June 1, 1997, and March 31, 1999 (2000). Hypertension was the single most frequent adverse effect (17 reports), followed by palpitations, tachycardia, or both (13); stroke (10); and seizures (7). Ten events resulted in death, and thirteen events produced permanent disability, representing twenty-six percent of the definite, probable, and possible cases.

Since dietary herbal supplements for obesity drugs are used for a stigmatized disease, there is the potential for abuse especially when they are available without a prescription. Thus, there is a reluctance to treat obesity with a supplement that has the potential for side effects. For both of these reasons there is a higher standard for the safety of obesity drugs than for other chronic diseases such as diabetes or hypertension (Greenway and Caruso, 2005). In fact, clinical trials for approval of obesity drugs are at least twice as long as the trials for diabetes or hypertension drugs.

There is also an economic challenge that obesity drugs have to face. Most insurance companies still consider obesity to be a cosmetic concern, and most obesity drug purchases are entirely covered by the consumer. Although consumers are concerned with their health, they are more concerned about looking thinner. Society is particularly prejudiced against women with obesity, and this is reflected in the percentage of women participating in clinical trials for obesity drugs. The prevalence of obesity is similar in both genders, but 80% of the participants in clinical trials for obesity drugs are women (Greenway and Caruso, 2005). Therefore, there is a

great challenge to meet the demand for a drug with a greater safety and efficacy than the present obesity drugs available.

Cosmetic Treatments for Obesity

Although the focus for medical issues associated with excess body fat is on people with a BMI over 30, even overweight people experience discrimination. Thus, the quality of life for the obese and overweight is impaired in our society. Treatments that allow safe body contouring and spot fat reduction have the potential to improve the quality of life experienced by the overweight and obese. Furthermore, safe medical treatments addressing cosmetic fat reduction may also reduce the demand for plastic surgical intervention, saving lives by reducing the need for more invasive treatments.

Fat Reduction

Fat is distributed into two different patterns, gynoid, or pear shaped, and android, or apple shaped. A major cosmetic concern for women with a gynoid fat distribution is the size of their thighs, while for women and men with an android fat distribution; it is the size of their waist. The influence of endogenous stimulators and inhibitors of the lipolytic process determine the threshold for lipolysis at the fat cell in the human body. The relative lipolytic thresholds of the body's fat cells, therefore, determine a person's fat distribution. Since obesity presents a real stigma for society, cosmetic alternatives have been created to treat the problem. Lipolytic stimulators have been injected locally or transdermally using ointments or creams (Greenway et al., 1995). Aminophylline 0.5% cream has been shown to reduce thigh girth compared to a vehicle control (Greenway et al., 1995). Aminophylline, two theophylline molecules joined by ethylenediamine, inhibits the breakdown of cyclic-AMP in the fat cell amplifying the lipolytic signal and lowering the lipolytic threshold. This study performed by Greenway et al. illustrates that aminophylline, a lipolytic stimulator, results in fat loss from a particular area when applied

locally to the thighs (1995). Aminophylline cream offers a safe and effective method for cosmetic local fat reduction from the thigh, and it is less invasive than cosmetic surgery.

Topical fat reduction from the thigh in women using aminophylline cream has been demonstrated, but the local fat reduction in other body areas or in men by lowering the lipolytic threshold with aminophylline cream has not. Since thigh fat reduction with aminophylline cream occurs due to a reduction in the local lipolytic threshold, the same principle should apply to other local body areas. Therefore, the waistline is another problematic area that will be tested to determine if aminophylline cream will reduce waist circumference compared to a control.

Fat Redistribution

When weight is stable, fat is stored throughout the day by food intake and used at night to uphold a balance in energy. Fat is distributed based on the sensitivity of the receptors located on the fat cells for lipolysis. Lowering the lipolytic threshold locally in one particular area and blocking lipolysis in another should result in fat redistribution. Pear-shaped women are often not only concerned about the amount and appearance of the fat on their hips and thighs, but they often also want larger breasts. Therefore, if the lipolytic threshold of the breast fat was raised while mobilizing fat from the thighs, one could, in theory, shift fat from the thighs to the breasts. This has more than theoretical interest, since women with these cosmetic concerns are now resorting to invasive procedures like liposuction and breast implants. If a combination of creams applied to the thighs and breasts were effective in shifting fat from one area to the other, this would be an alternative cosmetic solution, one that would be non-invasive and reversible with discontinuation of the creams.

Nicotinic acid is a vitamin that has been used in doses of two to five grams per day orally to treat elevations in cholesterol and triglycerides. Nicotinic acid reduces serum triglycerides by blocking lipolysis at the fat cell through inhibition of hormone sensitive lipase. This anti-

lipolytic effect involves the inhibition of cyclic adenosine monophosphate through an inhibitory G-protein acting upon adenylyl cyclase (Tunaru et al., 2003). A two gram oral dose of nicotinic acid gives a serum concentration of 10^{-4} Molar while the dose needed to maximally inhibit lipolysis in vitro is 10^{-5} Molar (Niaspan®, 2003 and Green et al., 1991). Therefore, injecting 10^{-5} Molar nicotinic acid into the breast would expose the fat cells in that area to levels only one tenth of the concentrations present in the blood stream during routine treatment of hypertriglyceridemia with nicotinic acid.

Utilizing the concept of "shifting fat", a study was performed to test the concept of fat redistribution since local fat reduction was effective. One woman used 0.5% aminophylline cream to both thighs and nicotinic acid cream to one breast twice a day for four weeks. There appeared to be a slight increase in the size of the treated breast, but the results of this pilot trial were in question. The breast measurements were done using water displacement. This proved to be a difficult method to use and one with irreproducible results since breasts float. It was concluded from this one patient pilot study that the development of a reproducible measure of breast volume was needed to prove the validity of the concept of shifting fat between body depots.

Mesotherapy

Mesotherapy is the process of injecting small amounts of drugs in the subcutaneous and intradermal tissues of the skin, the mesoderm, and was introduced by Dr. Michael Pistor in 1952 as a treatment for asthma (Pistor, 1964). Mesotherapy first began in France and the French medical schools actually include it in their program of study. Mesotherapy has recently been gaining in popularity in the United States. The primary application in the United States has been to induce lipolysis for local fat reduction and to reduce the appearance of cellulite on the buttocks and thighs of many women.

Isoproterenol, aminophylline, yohimbine, and melilotus are common ingredients used empirically in mesotherapy, alone and in combination. The use of mesotherapy solutions to induce local lipolysis is primarily based on empirical observations and long-tem clinical use, including the use of local anesthetics like lidocaine or procaine in these mesotherapy solutions. Therefore, it is important to determine the capacity of various mesotherapy compounds and mixtures to stimulate lipolysis as well as to determine the effect of combining a local anesthetic on lipolysis included in mesotherapy combinations.

CHAPTER 2

[*]TOPICAL FAT REDUCTION FROM THE WAIST

Background

The influence of endogenous stimulators and inhibitors of the lipolytic process determine the threshold for lipolysis at the fat cell in the human body. The relative lipolytic thresholds of the body's fat cells, therefore, determine a person's fat distribution. The greater abundance of lipolytically inhibitory alpha-2-adrenergic receptors on the thigh fat cells of women under the influence of estrogen is felt to be responsible for the characteristic lower body fat distribution typical for women (Lafontan et al., 1979). Fat is a dynamic storage organ. When weight is stable, fat is stored in fat cells during the day as people eat and used during the night to sustain the body until breakfast the next morning. Fat is quantitatively mobilized from the individual fat cells in proportion to the individual lipolytic thresholds of the regional fat deposits. It is, therefore, logical to assume that lowering the lipolytic threshold in a local fat deposit will cause its fat stores to be preferentially depleted through the dynamic process of body lipid turnover.

There are several methods to lower the local lipolytic threshold at the fat cell. One can inject a lipolytic stimulator locally or deliver the lipolytic stimulator transdermally using ointments or creams (Greenway et al., 1995). Aminophylline 0.5% cream has been shown to reduce thigh girth compared to a vehicle control (Greenway et al., 1995). Aminophylline, two theophylline molecules joined by ethylenediamine, inhibits the breakdown of cyclic-AMP in the fat cell amplifying the lipolytic signal and lowering the lipolytic threshold.

Fat is distributed in two different patterns – "gynoid" and "android". A major cosmetic concern for women with a gynoid fat distribution is the size of their thighs, while for women and men with an android fat distribution; it is the size of their waist. Since thigh fat reduction with

[*] Reprinted by permission of *Diabetes, Obesity and Metabolism.*

aminophylline cream occurs due to a reduction in the local lipolytic threshold, the same principle should apply to other local body areas. This study is designed to test the hypothesis that 0.5% aminophylline cream applied to the waist will cause preferential fat loss from that location.

Methods

Fifty overweight and obese men and women with a BMI > 27 kg/m^2, between the ages of 21 and 65 years of age and with an android fat distribution characterized by a waist to hip ratio $\geq$ 0.80 in women or $\geq$ 1.0 in men were included in this study. Subjects using aminophylline, theophylline, or having a known allergy to either was excluded, as were subjects with uncontrolled hypertension.

At baseline all subjects were instructed to follow a balanced 1200 kcal/d diet and encouraged to follow a walking program throughout the 12-week study. The subjects gave dietary histories of eating 1800-2200 kcal/d. Although we suspect that they may have been eating more, we chose a 1200 kcal/d diet since it is generally recognized as safe in a weight loss program and is a 600-1000 kcal/d deficit from their stated caloric intakes. The subjects were randomized with blocking for gender into two groups of 25 subjects, one receiving a 0.5% aminophylline water-based cream, and the other receiving no topical treatment served as a control. Randomization procedures were performed by a person who did not see the participants, using a random number table and blocking for gender. All participants in the 0.5% aminophylline cream group were instructed to rub 15 cc of the cream on their waist twice a day for the duration of the 12-week trial. For purposes of cream application, the waist was defined as the area from the lower rib margins to the iliac crest. Subjects were seen every 2 weeks, encouraged to follow their diet, encouraged to continue their walking program, encouraged to apply the cream twice daily, blood pressure and pulse were measured, the waist skin was inspected, and they were asked if they had experienced any adverse events. Each month, blood

was drawn to measure the theophylline level. The theophylline was measured at 6 and 24 hours after the cream application. The timing of levels was based on the oral absorption characteristics of aminophylline, and the standard theophylline assay used in the local clinical laboratory was employed for measurement. The BMI, waist circumference and hip circumference were re-measured at the end of the 12-week study. The BMI, waist circumference and the waist to hip ratio were analyzed by t-test.

Results

Twenty females and 5 males were in both groups, and groups were well matched for body mass index at baseline, 28.2 kg/m^2 $\pm$ 1.0 (SEM) vs. 28.5 $\pm$ 1.0 kg/m^2 (p = NS), range 27.1-35 kg/m^2, for the aminophylline cream and the control groups, respectively. The average waist circumference was 97.8 $\pm$ 2.4 cm, 93.8 $\pm$ 1.5 cm in women (range 80-105 cm) and 115 $\pm$ 3.6 cm in men (98-130 cm) confirming that the study population had an android fat distribution. The waist circumference of the two groups was not different at baseline. The average waist to hip ratio was 0.94 $\pm$ 0.03, 0.97 $\pm$ 0.001 in women (range 0.94 – 0.99) and 1.34 $\pm$ 0.17 (range 1.15-1.62). All 50 subjects completed the study.

At week 12, the BMI in the aminophylline cream group was 26.1 $\pm$ 1.0 kg/m^2 and 26.2 $\pm$ 1.0 kg/m^2 in the control group, a significant reduction in BMI from baseline of 2 kg/m^2 in both groups. The reduction in waist circumference was 11 $\pm$ 1.0 cm in the aminophylline cream group and 5.0 $\pm$ 0.6 cm in the control group ($p<0.001$). The reduction in waist circumference was significant for both women (11.6 $\pm$ 0.6 cm in the aminophylline group and 5.6 $\pm$ 0.6 cm in the control group) and men (9.4 $\pm$ 0.7 cm in the aminophylline group and 4.7 $\pm$ 0.6 cm in the control group) ($p<0.001$). Women treated with 0.5% aminophylline cream lost more girth (11.6 $\pm$ 0.6 cm vs. 9.4 $\pm$ 0.7 cm) than the men ($p<0.001$), see Table 2.1. The waist to hip ratio declined more in the group treated with 0.5% aminophylline cream than the control group (0.08 $\pm$ 0.03 vs.

0.02 ± 0.03, p<0.01). The waist to hip ratio in women declined more in the group treated with 0.5% aminophylline cream than the control group (0.12 ± 0.001 vs. 0.05 ± 0.001, p<0.001), and the waist to hip ratio in men declined more in the group treated with 0.5% aminophylline cream than the control group, but this difference was not statistically significant (0.2 ± 0.06 vs. 0.09 ± 0.07, NS), see Table 2.2. The lack of difference in weight loss between the two groups and the difference in waist circumference suggests a cosmetic change in fat distribution. All monthly aminophylline levels were undetectable. There were no adverse events or allergic reactions to the cream. Blood pressure and pulse remained in the normal range throughout the study.

Table 2.1. Changes in Waist Circumference (cm) from Baseline in the Two Groups and Separately for Men and Women

Group	Waist	Male Waist	Female Waist
0.5% aminophylline (cm)	-11 ± 1.0[a]	-9.4 ± 0.7[c]	-11.6 ± 0.6[a]
Control (cm)	-5.0 ± 0.6[b]	-4.7 ± 0.8[b]	-5.6 ± 0.6[b]

Table 2.1 shows the changes in waist circumference (cm) from baseline in the two groups and separately for men and women. Different superscripts show values that are significantly different from one another (p<0.001).

Table 2.2. Changes in Waist to Hip Ratio (cm) from Baseline in the Two Groups and Separately for Men and Women

Group	Waist/Hip	Male Waist/Hip	Female Waist/Hip
0.5% aminophylline (cm)	-0.08 ± 0.03^{e}	-0.2 ± 0.06	-0.12 ± 0.001^{g}
Control (cm)	-0.02 ± 0.03^{f}	-0.09 ± 0.07	-0.05 ± 0.001^{h}

Table 2.2 shows the changes in waist to hip ratio from baseline in the two groups and separately for men and women. Different superscripts show values that are significantly different from one another ($p<0.001$).

Discussion

This trial demonstrates that reducing the local lipolytic threshold with topical aminophylline cream results in a reduction of waist circumference in both men and women. Local girth reduction of the waist in android body types is consistent with the principle that lowering the local lipolytic threshold causes fat reduction in the area of application. Thus, one can extend the principle of local fat reduction with aminophylline cream to both genders and to a body area different from the thigh. This difference in waist circumference with a similar weight loss in the two groups suggests a cosmetic redistribution of body fat.

In developing aminophylline cream it was appreciated that aminophylline, two theophylline molecules joined by ethylenediamine, is a skin sensitizer and chemically reactive due to the ethylenediamine it contains. A standard cream base turned yellow from a chemical reaction with aminophylline. This yellow cream was ineffective and caused rashes in some subjects.

Using a specially formulated cream base to stabilize the aminophylline, the safety and efficacy of local thigh fat reduction was demonstrated (Greenway et al., 1995). The same cream base was used in this study. Not only was the aminophylline cream effective for reduction of waist circumference in this study, but also it was safe. The undetectable aminophylline levels confirmed that the cream was acting locally, and there were no rashes or adverse events during the trial.

Although a placebo cream was not used in the control group, the two groups were well matched at baseline, and the BMI loss was similar in both groups. The purpose of the 1200 kcal/d weight loss diet was twofold: 1) to address the subject's overweight problem, and 2) to lower the lipolytic threshold through negative caloric balance.

The waist to hip ratio has been used as a surrogate measure of insulin resistance due to its correlation with visceral fat (Peiris et al., 1987 and Peiris et al., 1988). Visceral fat correlates with insulin resistance (Sironi et al, 2004). Since the local fat reduction reduced the subcutaneous abdominal fat, it presumably does not reduce insulin resistance despite the reduction in the waist to hip ratio. The change in waist to hip ratio in this study is confirmation that the fat shifted away from the waist.

The most common cosmetic concern for those with a gynoid fat distribution is the size of their thighs, and for those with the android fat distribution is the size of their waist. This study demonstrates that fat can be preferentially and safely mobilized from the waist during weight loss in those with an android fat distribution. Psychosocial benefit may derive from the cosmetic changes, but these were not addressed in this study. Aminophylline cream offers a safe and effective method for cosmetic local fat reduction from the waist, and it is less invasive than cosmetic surgical procedures.

CHAPTER 3

*THE COST-EFFECTIVENESS OF THREE DIFFERENT MEASURES OF BREAST VOLUME

Introduction

Our clinical research program is preparing to test topically applied compounds to increase breast size as a medical substitute for surgical breast augmentation surgery. In order to evaluate the efficacy of breast augmentation creams, one needs a validated and reproducible method of measuring breast size. Since women with small breasts most frequently request breast augmentation, any measuring procedure must be capable of measuring very small breast sizes. Previously used breast measuring systems have focused upon breast sizes near the mean, and the applicability of these measurement systems to small breasts is not known.

Breast sizing for bra fitting has used the difference in chest circumference measured at the ribcage near the submammary crease and the circumference at the nipple level. Although the precision of this measure may be adequate as a starting point to fit bras in a women's underwear shop, it is too imprecise to use as a scientific outcome measure of breast size, especially when the breasts are ptotic (Pechter, 1998).

The Grossman-Roudner Breast Measuring Device is a circle of flexible plastic with a cut to the center along a radius line. This circle, which can be formed into a cone-shaped device, comes in three diameters and is appropriate for measuring breasts with volumes of 125-200 cc, 200-300 cc, and 300-425 cc, which roughly correspond to A, B and C size breasts. The circle is overlapped upon itself to make a cone, and the cone is shaped around the breast with the volume of the cone determined at the overlap of the cut radius on the surface of the cone. The Grossman-Roudner Breast Measuring Device was formerly available from Cox-Uphoff International, in Santa Barbara, California.

---

* Reprinted by permission of *Aesthetic Plastic Surgery*.

The casting method involves exerting gentle pressure on the breast tissue. One can define the edges of the glandular tissue. The edges of the breast tissue can be marked by applying kite string to the chest wall with glue. Making a cast of the breast tissue and measuring the volume of the cast may be a more accurate method of measuring breast size than the Grossman-Roudner cone, since breasts are not truly conical in shape. MRI is a method that gives the greatest anatomical detail, and similar imaging techniques have been used to measure anatomic volumes such as intraabdominal fat (Bulstrode et al., 2001). Magnetic resonance imaging has been used to predict survival in individuals with cervical cancer by measuring the tumor volume (Soutter et al., 2004). Therefore, we thought that measuring breast volume using calculations from a magnetic resonance imaging (MRI) scan would be the most accurate and reproducible method, yet also the most costly.

This study proposes to compare the volume of the breast measured from the Grossman-Roudner Breast Measuring Device, the volume of a breast cast, and the volume of the breast measured from an MRI scan. These methods will be compared to determine the reproducibility of the measures over a range of breast sizes. The variability measure will be used to determine the number of subjects needed to detect a given difference in breast volume. The number of subjects multiplied by the cost of the test will be used to determine the relative cost-effectiveness of the three methods.

Methods

Five women who had no breast surgery were included in this study. Each woman represented one of the following bra cup sizes: AA, A, B, C, and D. Each woman had three separate breast volume measurements using the following three methods.

1. Grossman-Roudner Breast Measuring Device (Grossman and Roudner, 1980): The circular plastic measuring device is wrapped around the breast to form a cone, and the

volume is read off the overlap of the radius with the surface of the cone with the woman sitting and leaning backward at a 45 degree angle. A larger size was constructed for volumes of 450-600 cc corresponding to size D, adding to the standard sizes A, B, and C that come with the set sold commercially in the past.

2. Breast Casting: The woman lay in a semi-recumbant position, and gentle pressure was applied to the breast to define the margins of the glandular tissue. Rubber cement (like the glue used to make scrapbooks) was painted at the margins of the glandular tissue to attach kite string to the skin and mark the breast tissue margins. Strips of plaster of Paris impregnated gauze (like those used to make splints) were cut to the size of the anterior thorax, dipped in warm water and applied over the breast, completely covering the glandular tissue as well as the string.

   Three layers of plaster of Paris impregnated gauze were applied and allowed to harden. The cast of the breast along with the string was lifted from the chest. The string was then removed, leaving a groove in the Plaster of Paris defining the limits of the breast on the inner aspects of the cast. After further drying, the inner portion of the cast was sealed with rubber cement. A 50:50 fine sand and butter mixture was then placed in the cast and smoothed to the concavity of the chest wall. The volume of the sand-butter mixture was measured by water displacement in a graduated cylinder.

3. Magnetic Resonance Imaging: The women went for an MRI examination of their breasts. The MRI machine was made by Siemens and has a 1.5 tesla magnet. The volume of the breast tissue was determined from the MRI data files in the imaging laboratory using a procedure for quantifying visceral fat adapted to the quantification of breast volume. An attempt was made to include only the glandular tissue of the breast in each measure. The volume of each slice of the breast was determined. The whole breast

volume was calculated by adding the volumes of the individual slices together. MRI slices were 8.4 mm apart in the sagittal plane, and volume was calculated using the ANALYZE bio-imaging software.

Analysis

The mean and standard deviation of the three breast volume measures of the three methods on each subject were used to construct a power analysis determining the number of subjects needed to detect a 5% change in breast volume with 80% power and an alpha of 0.05. The number of subjects was multiplied by the cost of the test to determine the most cost effective method to use.

Results

Three measurements were made in each of the five subjects with each of the three measurement methods (Table 3.1). The mean and standard deviation of the mean were calculated for each subject using each of the three measurement methods. A power analysis was then performed to determine the number of subjects that would be needed to detect a 5% change in volume given these variances with 80% power and an alpha of 0.05. The number of subjects needed to detect a 5% change in each subject for each test was multiplied by the cost of the test in question. These costs to detect a 5% change in volume for each of the tests were compared over the range of breast sizes (Table 3.2).

Measuring the breast using the Grossman-Roudner cone takes about ten minutes to perform for a person getting paid minimum wage. Therefore, volumes with this device cost about $1.00 per measurement. The casting material is made of gauze impregnated with plaster of Paris. This measurement takes about one hour to perform the test and about one hour to measure the volume of the breast for a person getting paid minimum wage, which totals to about $12.00. The cost of the material used for each measurement, including the gauze impregnated

with plaster of Paris, kite string, butter, sand, and rubber cement is about $8.00. Therefore, measurements with this device cost about $20.00 per measurement.

The three MRI scans take about thirty minutes to capture each of the breast images. Measurements with this device cost about $1,400 for each MRI scan performed.

The ratio of relative costs to detect a 5% change in breast volume for the Grossman-Roudner cone, cast, and MRI is 1:64-189:373-33,500 (1:127:16,967) respectively. Since these ratio ranges do not overlap, the Grossman-Roudner Breast Measuring Device clearly is the most cost effective method for determining changes in breast volume in studies testing topical therapies to change breast size.

Table 3.1. Measurements of Cup Sizes AA, A, B, C, and D

Cup Size	AA	A	B	C	D
Cone (cc)	160	170	290	325	470
Cone (cc)	165	165	300	350	495
Cone (cc)	160	180	280	350	515
Cast (cc)	251	260	435	475	780
Cast (cc)	255	270	430	555	690
Cast (cc)	235	320	365	530	655
MRI (cc)	347.5	465	512	544	940
MRI (cc)	377.3	465	475	556	969
MRI (cc)	440.7	472	484	524	968

Table 3.2. Relative Number and Cost to Detect a 5% Change in Breast Volume

Cup Size	AA	A	B	C	D
Cone (mean in cc ± SD)	162 ± 3	171 ± 6	290 ± 10	342 ± 14	493 ± 23
N for 5% change	4	10	9	13	15
$ for 5% change	4	10	9	13	15
Cast	247 ± 11	283 ± 32	405 ± 33	539 ± 50	708 ± 64
N for 5% change	13	82	85	56	54
$ for 5% change	260	1,640	1,700	1,120	1,080
MRI	389 ± 48	467 ± 4	490 ± 19	541 ± 16	959 ± 16
N for 5% change	96	3	11	7	4
$ for 5% change	134,400	4,200	15,400	9,800	5,600

Discussion

Several methods have been proposed to measure breast volume, but no cost-effectiveness evaluation comparing methods has been done. Anatomical measures to fit brassieres, water displacement, casting, the Grossman-Roudner cone, photography, mammograms, ultrasound and magnetic resonance imaging (MRI) have all been suggested as methods to measure breast volume.

Anatomical measures such as those used for fitting brassieres are inaccurate for measuring breast volume due to variability in breast shape (Bulstrode et al., 2001). Cup size is typically determined by the difference in circumferences of the chest at the submammary crease and the nipple line. This measure varies considerably as breasts become ptotic. Brassiere sizes are also inaccurate since cup size varies with each brand.

Water displacement based on Archimedes principle is another way of determining the breast volume. The subjects had difficulty performing this test because the breast tissue, being composed of fat tissue, floated (Bulstrode et al., 2001). The variability of this measurement due to different levels of submergence was, therefore, common, and the measurement's wide variability made it inaccurate (Tezel et al., 2000).

The casting method for determining breast volume has been tested measuring both breasts at the same time. A study carried out by Campaigne et al. using plaster strips applied horizontally across both breasts to measure the volume of both breasts gave an error of $\pm$ 10.2%, which was due to variability creating the casts and variability filling the casts with sand, resulting in large within-subject variability (Campaigne et al., 1979). To observe the variability of filling the casts of the breast pairs with sand, the same person filled 30 randomly selected casts twice, 30 minutes apart. It was estimated that half of the error was due to filling the cast with sand. Campaigne et al., however, did find that the reproducibility improved after repeating the filling ten times (1979). In another study analyzing breast volume measurements executed by Bulstrode et al., thermoplastic sheets were used as the casting material, and only a single breast was measured (2001). Bulstrode noted that there were no distinguishable disadvantages in using the casting method to measure breast volume. One had a visual model of the breast to evaluate the shape, and the thermoplastic molding was a convenient and well-tolerated method for the subjects. Therefore, we decided to use a casting method for our study to determine breast volume using a single breast.

The Grossman-Roudner Breast Measuring Device has been developed as an easy, precise, and cost-effective method of determining breast volume (Grossman et al., 1980). This method has been compared with the casting method, and the Grossman-Roudner cone proved to be reproducible (Palin et al., 1986). Jack Grossman, the developer of the Grossman-Roudner

Breast Measuring Device stated that this method is "simple and direct and by no means absolute in its volume determination for breasts" (Palin et al., 1986). He further explained that this technique provides a relative reproducibility that is helpful for aesthetic purposes (Palin et al., 1986). As a result, we chose to utilize the Grossman-Roudner Breast Measuring Device for our study to measure breast volume.

Spectrophotography gives the volume measurements and the relative size relationship of normal breast pairs. We are interested only in breast volume. Costly equipment is necessary for performing the necessary stereophotogrammetry, and it is a very time-consuming process. In a study performed by Loughry et al. using this method, wide-angle stereometric cameras, a surface contrast optical projector, and a double-rail support were required (1987). The stereocameras were equipped with biogon lenses, vacuum film platens to insure film flatness, and fiber optic bundles that placed fiducial marks on the exposed film. In addition, the production of the total breast volumes for the subjects required the use of several mathematical algorithms. A drawback to using photography as a breast volume measurement is the skill required by the person using the stereometric cameras, the cost of the associated equipment, and necessary expertise in the mathematical algorithms.

Mammography, ultrasound, and MRI use similar principles in measuring the volume of the breast by indirect visualization techniques. Breast volumes determined by mammography have been demonstrated to have good correlation with breast volumes measured at a subsequent mastectomy to remove a malignant tumor (Bulstrode et al., 2001). Mammography, however, involves the risk of radiation exposure that is difficult to justify for cosmetic applications. Mammography is also more uncomfortable for the subject than the other measurement methods in this group (Bulstrode et al., 2001). Although ultrasound and MRI use a similar principle to measure breast volume, the use of magnetic resonance gives much better separation of tissue

planes and better definition of the breast tissue than the use of ultrasound. Therefore, we chose to evaluate magnetic resonance as the best measurement to represent this group of methods.

Of the various methods to measure breast volume we selected two anatomic measures and one indirect measure to evaluate as having the best qualities for cosmetic applications: the Grossman-Roudner cone, breast casting and magnetic resonance imaging. We expected that the magnetic resonance imaging would give the most accurate and precise estimation of breast volume. We expected that the Grossman-Roudner cone would not have sufficient accuracy due to the device not containing the entire breast tissue within the cone. We expected that the breast casting would be the most cost-effective measure, since it measured the entire breast and is much less expensive than magnetic resonance imaging. To our surprise, the Grossman-Roudner cone was clearly the most cost-effective measure. Despite the fact that the entirety of the breast is not contained in the cone during measurement, the reproducibility of the measure was comparable to the magnetic resonance imaging. Therefore, the Grossman-Roudner cone appears to be the most cost effective and preferred measure for estimating change in breast volume during a cosmetic treatment program to alter breast size.

CHAPTER 4

AN EVALUATION OF MESOTHERAPY SOLUTIONS FOR INDUCING LIPOLYSIS AND TREATING CELLULITE

Background

Michel Pistor gave an intravenous injection of procaine to a man suffering from asthma in France during the 1950's, and this experience started the practice of mesotherapy. Although the asthma did not improve, the man's long-standing deafness improved temporarily. Dr. Pistor concluded that injection into the subcutaneous tissues would give the best results, and claimed many health benefits from his method of injecting procaine locally. Since the tissue into which he was injecting came from mesodermal origin, he called this technique mesotherapy (Pistor, 1964).

Mesotherapy has gained popularity in France and is now included in French medical school curricula. Mesotherapy has recently been gaining in popularity in the United States. The primary application of mesotherapy in the United States has been to induce lipolysis for local fat reduction and to reduce the appearance of cellulite, the lumpy-bumpy, orange-peel texture of the skin on the buttocks and thighs of many women. The use of mesotherapy solutions to induce local lipolysis is primarily based on empirical observations and long-term clinical use, including the incorporation of local anesthetics like lidocaine or procaine in these mesotherapy solutions. This study evaluates the lipolytic potential of several compounds commonly used separately and together in cosmetic mesotherapy solutions using a human fat cell assay with the generation of glycerol as the measure of lipolysis.

Methods

The lipolysis assay (ZenBio, Research Triangle Park, North Carolina) uses differentiated human adipocytes, which are adherent to the bottom of each well in a 96 well plate to test the effects of various compounds on lipolysis. The test compounds will either stimulate or inhibit

the release of glycerol from the adipocytes relative to the assay buffer, and this difference is stated as a fold induction index. Controls of assay buffer, saline and positive controls of isoproterenol and isobutylmethylxanthine (IBMX) were also included. Table 4.1 lists the mesotherapy solutions tested and the concentrations at which they were tested.

Upon arrival of the lipolysis assay kit, 100 ul of the shipping medium was removed from each well and discarded. Plate A was placed in the incubator for 5-7 days to allow the cells to recover from the stress of shipping. Prior to removing Plate A from the incubator, all test compounds were made by diluting them to their final concentration in Assay Buffer. On the day of the assay, 150 ul of media was removed from each well of Plate A, and 200 ul of Wash Buffer was then added to each well of Plate A. Next 200 ul of media and Wash Buffer was removed, and another 200 ul of Wash Buffer was added to each well of Plate A. All media and Wash Buffer were removed from each well of Plate A. The cells were treated with 150 ul of the test compounds and controls, three wells at a time. The diluted IBMX and isoproterenol were treated as positive controls, and the assay buffer was treated as one of the vehicle controls. The plate was incubated for five hours at 37 degrees Celsius, 5% CO_2.

After incubation, 100ul of the test compounds were removed and added to a sterile 96 well plate. 100ul of glycerol reagent were then added to create a colorimetric assay dependent on the amount of glycerol released during incubation with the test compounds. A series of glycerol standards were also run with each assay to create a standard curve upon which the results were based. The plates were then read in a spectrophotometer plate reader at 540nm and the results were plotted based on the standard curve.

Results

Isoproterenol, aminophylline, yohimbine (Figure 4.1) and melilotus all significantly stimulated lipolysis compared to the buffer control. The addition of aminophylline to melilotus

significantly increased lipolysis compared to melilotus alone (Figure 4.2). The addition of aminophylline to isoproterenol increased lipolysis significantly compared to isoproterenol alone (Figure 4.3). The addition of lidocaine to isoproterenol and aminophylline (Figure 4.4) inhibited lipolysis such that the combination was not statistically different than control. The addition of lidocaine to the combination of isoproterenol, aminophylline and yohimbine (Figure 4.5) inhibited lipolysis but the combination was still statistically different than control ($p<0.05$).

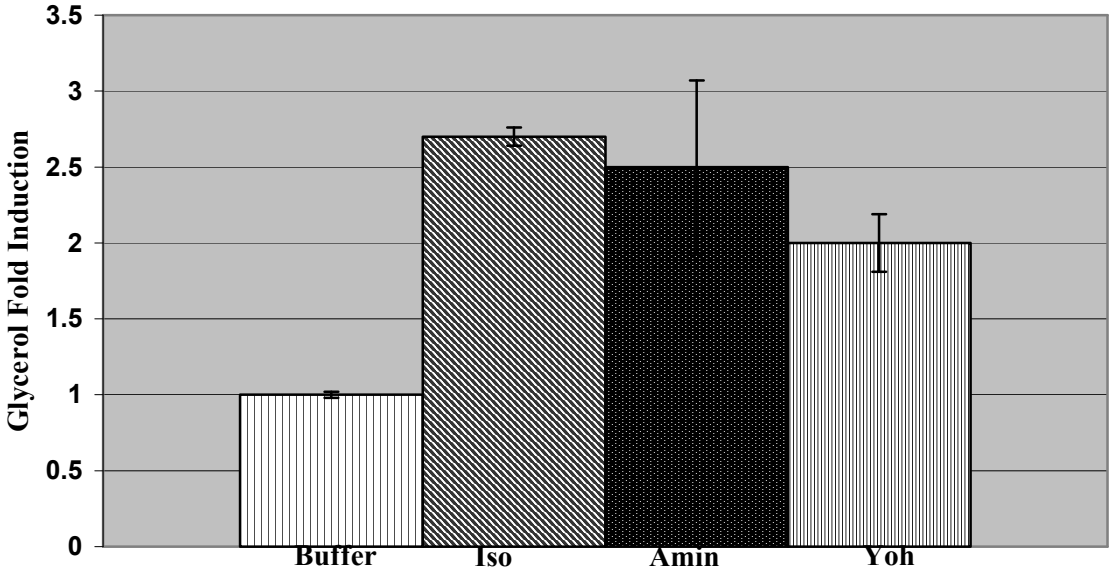

Figure 4.1. Lipolysis Fold Induction Compared to Assay Buffer

Abbreviations: Iso – Isoproterenol, Amin – Aminophylline, Yoh – Yohimbine

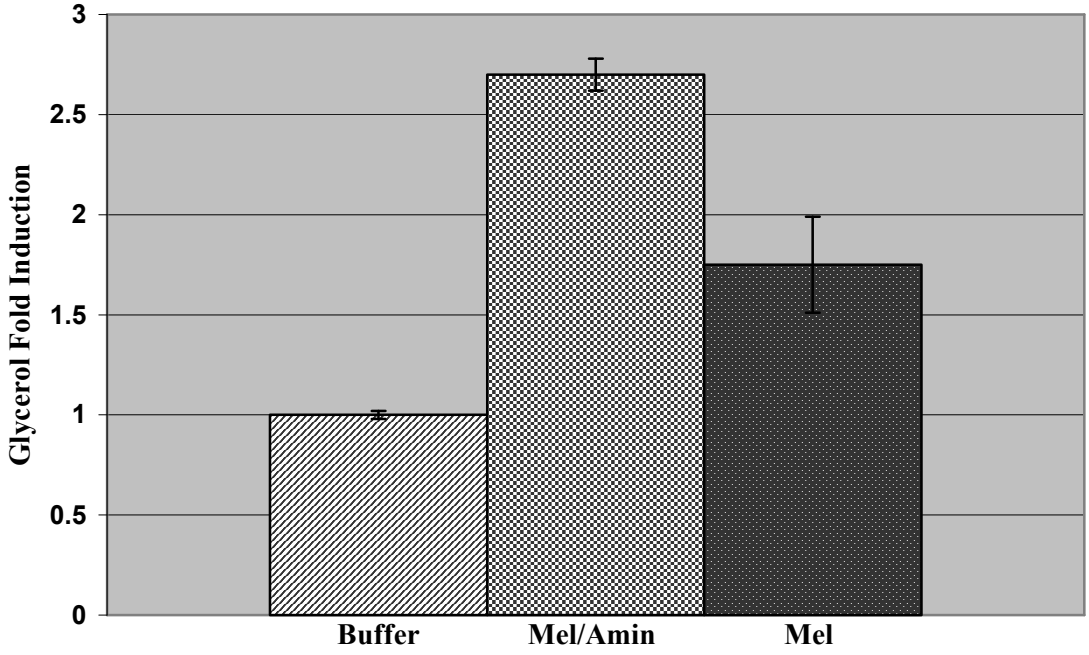

Figure 4.2. Lipolysis Fold Induction Compared to Assay Buffer

Abbreviations: Mel – Melilotus, Amin – Aminophylline

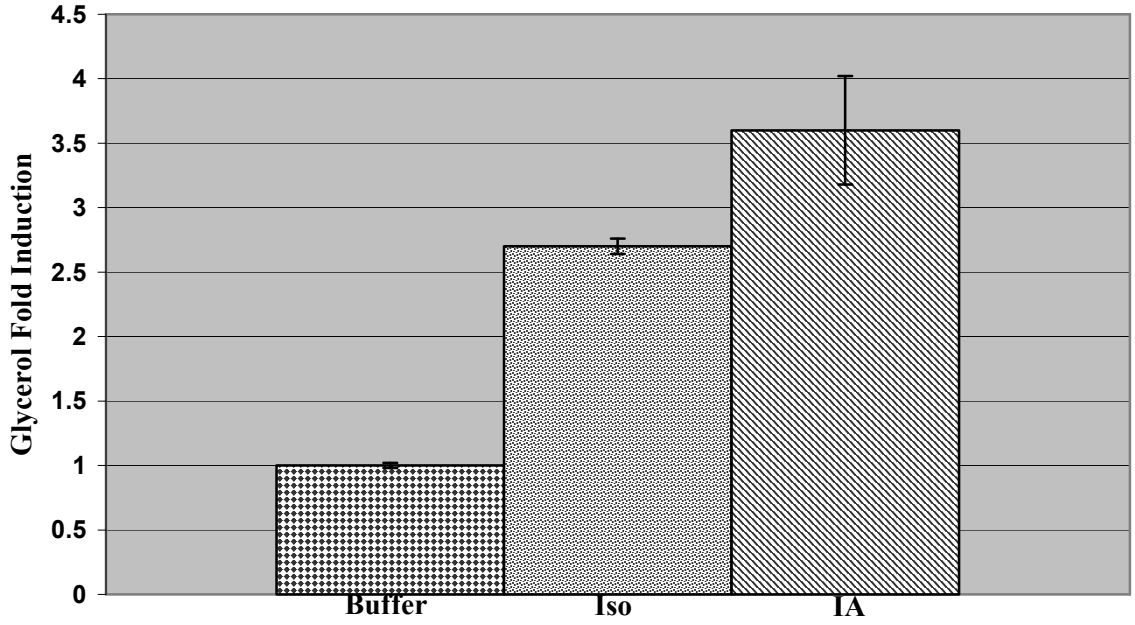

Figure 4.3. Lipolysis Fold Induction Compared to Assay Buffer

Abbreviations: Iso – Isoproterenol, IA -Isoproterenol, Aminophylline

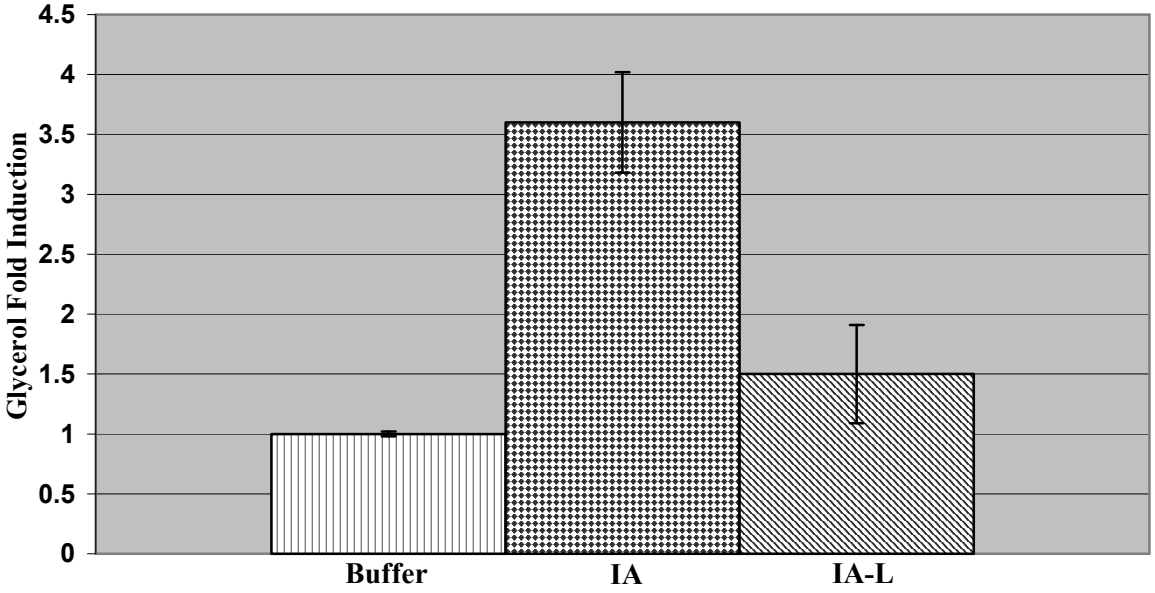

Figure 4.4. Lipolysis Fold Induction Compared to Assay Buffer

Abbreviations: IA – Isoproterenol, Aminophylline, IA-L – Isoproterenol, Aminophylline, and Lidocaine

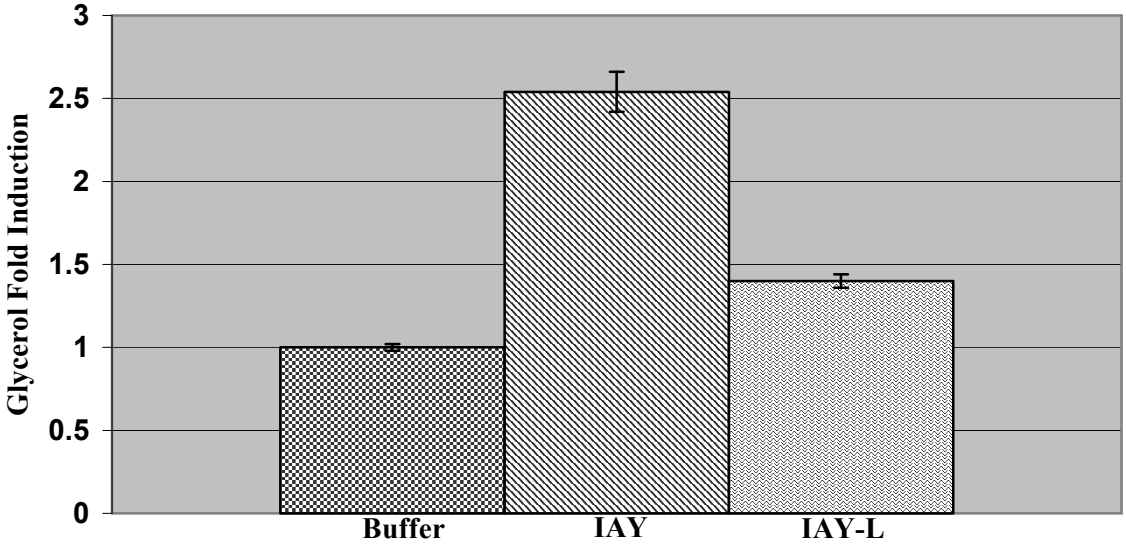

Figure 4.5. Lipolysis Fold Induction Compared to Assay Buffer

Abbreviations: IAY – Isoproterenol, Aminophylline, and Yohimbine, IAY-L – Isoproterenol, Aminophylline, Yohimbine, and Lidocaine

Table 4.1. Stimulation of lipolysis by compounds used in mesotherapy

Components	Concentration	Fold Induction	p-value
IBMX Control	1.0 x 10^{-4} M	2.3 $\pm$ 0.24	$p<0.01$
Assay Buffer	full strength	1.0	
Aminophylline	1.0 x 10^{-4} M	2.5 $\pm$ 0.57	$p<0.00004$
Isoproterenol	1.0 x 10^{-7} M	2.7 $\pm$ 0.06	$p<0.002$
Yohimbine	1.0 x 10^{-7} M	2.0 $\pm$ 0.19	$p<0.001$
Melilotus	0.02%	2.2 $\pm$ 0.33	$p<0.01$
Melilotus Aminophylline	0.02% 1.0 x 10^{-4} M	2.7 $\pm$ 0.05	$p<0.001$ vs. control $p<0.001$ vs. melilotus

Table 4.1 Continued

Aminophylline Isoproterenol	1.0 x 10^{-4} M 1.0 x 10^{-7} M	3.6 $\pm$ 0.42	p<0.001 vs. control p<0.01vs.Isopro.
Aminophylline Isoproterenol Yohimbine	1.0 x 10^{-4} M 1.0 x 10^{-7} M 1.0 x 10^{-7} M	2.5 $\pm$ 0.12	p<0.0007 vs. control
Aminophylline Isoproterenol Lidocaine	1.0 x 10^{-4} M 1.0 x 10^{-7} M 1.0 x 10^{-5} M	1.5 $\pm$ 0.41	p=NS vs. control
Aminophylline Isoproterenol Yohimbine Lidocaine	1.0 x 10^{-4} M 1.0 x 10^{-7} M 1.0 x 10^{-7} M 1.0 x 10^{-5} M	1.4 $\pm$ 0.04	p<0.05 vs. control

Discussion

The most important finding of this study was the inhibition of lipolysis by topical anesthetics, since almost all mesotherapy solutions have routinely included procaine, lidocaine or some other topical anesthetic. Evidence, however, exists in the medical literature that local anesthetics do inhibit lipolysis in human fat cells. Mersmann demonstrated lipolytic inhibition with procaine (1983), and others have shown that procaine uncouples adenylate cyclase from activating hormone sensitive lipase, the lipolytic enzyme in fat cells (D'Costa et al., 1979). The inhibition of lipolysis by procaine is shared by lidocaine (Komabayashi et al., 1978), and since another topical anesthetic, prilocaine, also inhibits lipolysis, this seems to be a class effect of local anesthetics (Arner et al., 1973).

Melilotus is an extract of sweet clover that has been used empirically in mesotherapy to induce lipolysis either alone or in the presence of aminophylline. Our studies confirm that melilotus used alone stimulates lipolysis and that the addition of aminophylline further increases lipolysis.

Isoproterenol, yohimbine and aminophylline are all well recognized lipolytic stimulators (Greenway et al., 1995). This study just served to confirm that finding. Isoproterenol has been reported to give fat loss on one thigh using mesotherapy injections compared to saline injections on the opposite thigh. Although there are no mesotherapy injection studies demonstrating the clinical efficacy of the other lipolytic stimulators to induce local fat reduction, there are studies using an ointment containing yohimbine, forskolin and aminophylline, an ointment with forskolin alone, an ointment with aminophylline alone and a cream with aminophylline alone in which the treated thigh lost significantly more girth that the thigh treated with placebo (Greenway et al., 1995 and Greenway and Bray, 1987). This suggests that delivery of lipolytic agents locally whether by injection or by topical application will cause local fat loss.

Mesotherapists have traditionally combined lipolytic stimulators with the goal of enhancing lipolysis to a greater extent by the use of polypharmacy. Our studies demonstrate that lipolysis induced by melilotus is enhanced by aminophylline as is the lipolysis induced by isoproterenol. Although confirmation will require clinical trials, the enhanced lipolysis in the fat cell assay suggests that combining lipolytic stimulators will enhance local fat reduction in mesotherapy practice.

Cellulite is the descriptive name of the lumpy-bumpy, or cottage cheese-appearance that is apparent on the thighs of some women. This appearance of the fat is felt to be the result of the connective tissue architecture of subcutaneous fat with women having connective tissue oriented at right angles to the skins surface as opposed to men who have a more diagonal orientation of this connective tissue (Pierard et al., 2000). Querleux agrees that women with cellulite have connective tissue oriented at right angles to the skin surface and describes an increase in the deeper adipose layer below Camper's fascia in women with cellulite, but disputes Pierard's depiction of the orientation of male connective tissue (Querleux et al., 2002). Since the

appearance is caused by fat cells bulging against these connective tissue strands, emptying out these fat cells by stimulating lipolysis will improve the smoothness of the skin surface. This is a desirable cosmetic effect for many women, and the treatment of cellulite is a major focus of mesotherapy.

Since injecting isoproterenol in the thighs reduces thigh girth and reduces the appearance of cellulite, injection of other lipolytic substances should cause local fat reduction and improve the appearance of cellulite as well. We have confirmed the lipolytic activity of melilotus, aminophylline, yohimbine and isoproterenol in a human fat cell assay, ingredients which are used for local fat reduction and to reduce the appearance of cellulite in the practice of mesotherapy. We have also demonstrated that combinations of these lipolytic stimulators used in the practice of mesotherapy give greater stimulation of lipolysis than the individual components alone. Most importantly, we confirmed earlier reports in the literature showing that lidocaine and other topical anesthetics inhibit lipolysis. We believe that local anesthetics such as lidocaine and its class derivatives should be removed from mesotherapy solutions designed to cause local fat reduction and reduce the appearance of cellulite.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The first study confirmed that aminophylline cream application to the waist will reduce waist circumference compared to a control. At week twelve, the BMI in the aminophylline cream group was 26.1 $\pm$ 1.0 kg/m^2 and 26.2 $\pm$ 1.0 kg/m^2 in the control group, a significant reduction in BMI from baseline of 2 kg/m^2 in both groups. The reduction in waist circumference was 11 $\pm$ 1.0 cm in the aminophylline cream group and 5.0 $\pm$ 0.6 cm in the control group ($p<0.001$). The reduction in waist circumference was significant for both women (11.6 $\pm$ 0.6 cm in the aminophylline group and 5.6 $\pm$ 0.6 cm in the control group) and men (9.4 $\pm$ 0.7 cm in the aminophylline group and 4.7 $\pm$ 0.6 cm in the control group) ($p<0.001$). Women treated with 0.5% aminophylline cream lost more girth (11.6 $\pm$ 0.6 cm vs. 9.4 $\pm$ 0.7 cm) than the men ($p<0.001$). The waist to hip ratio declined more in the group treated with 0.5% aminophylline cream than the control group (0.08 $\pm$ 0.03 vs. 0.02 $\pm$ 0.03, $p<0.01$). The waist to hip ratio in women declined more in the group treated with 0.5% aminophylline cream than the control group (0.12 $\pm$ 0.001 vs. 0.05 $\pm$ 0.001, $p<0.001$), and the waist to hip ratio in men declined more in the group treated with 0.5% aminophylline cream than the control group, but this difference was not statistically significant (0.2 $\pm$ 0.06 vs. 0.09 $\pm$ 0.07, NS). The lack of difference in weight loss between the two groups and the difference in waist circumference suggests a cosmetic change in fat distribution. All monthly aminophylline levels were undetectable. There were no adverse events or allergic reactions to the cream. Blood pressure and pulse remained in the normal range throughout the study.

This trial demonstrates that reducing the local lipolytic threshold with topical aminophylline cream results in a reduction of waist circumference in both men and women. Local girth reduction of the waist in android body types is consistent with the principle that

lowering the local lipolytic threshold causes fat reduction in the area of application. Thus, the principle of local fat reduction with aminophylline cream can be applied to both genders and to a body area different from the thigh. This difference in waist circumference with a similar weight loss in the two groups suggests a cosmetic redistribution of body fat.

The second study developed a cost-effective method of breast measurement that will allow the concept of fat redistribution to be tested. Anatomical measures to fit brassieres, water displacement, casting, the Grossman-Roudner cone, photography, mammograms, ultrasound, and MRI have all been suggested as methods to measure breast volume, but no cost-effectiveness evaluation comparing methods has been done. Breast volume measurements using the Grossman-Roudner measuring device, plaster casting, and MRI were compared. It was predicted that the magnetic resonance imaging would give the most accurate and precise estimation of breast volume since it is the gold standard. It was expected that the Grossman-Roudner cone would not have sufficient accuracy due to the device not containing the entire breast tissue within the cone. It was projected that the breast casting would be the most cost-effective measure, since it measured the entire breast and is much less expensive than magnetic resonance imaging. Surprisingly, the Grossman-Roudner cone was clearly the most cost-effective measure. Despite the fact that the entire breast tissue is not contained in the cone during measurement, the reproducibility of the measure was comparable to the magnetic resonance imaging. Therefore, the Grossman-Roudner cone appears to be the most cost effective and preferred measure for estimating change in breast volume during a cosmetic treatment program to alter breast size.

The third study demonstrates that single mesotherapy drugs used in mesotherapy solutions increase lipolysis, and the combination solutions increase lipolysis greater than the individual ingredients. This study also shows that lidocaine and other topical anesthetics inhibit lipolysis of mesotherapy solutions. The following single drugs and herbs were tested and

elevated lipolysis by the following multiples of the glycerol generation attributable to the assay buffer: isoproterenol 10^{-7} M: 2.7 times, aminophylline 10^{-4} M: 2.5 times, yohimbine 10^{-7} M: 2.0 times, melilotus extract 0.02%: 2.2 times, but triac, a derivative of thyroid hormone did not stimulate lipolysis. The following combinations increased glycerol generation by the following multiples of the assay buffer control: isoproterenol and aminophylline: 3.6 times, isoproterenol, aminophylline and yohimbine: 2.5 times, melilotus and aminophylline: 2.7 times.

The addition of lidocaine to either of the first two combinations blocked lipolysis. Lidocaine and procaine both block lipolysis by uncoupling the increase of cyclic-AMP inside the fat cell from hormone sensitive lipase activation. It is concluded that with the possible exception of triac, the single mesotherapy drugs or homeopathic ingredients used in mesotherapy solutions that we tested increase lipolysis. Combination mesotherapy solutions increase lipolysis more than the individual ingredients. Lidocaine and other local anesthetics should be removed from mesotherapy solutions because they block lipolysis.

These studies set the stage for our future research. We have demonstrated that mesotherapy is an effective method for proof of concept studies to redistribute local fat deposits for cosmetic purposes. Studies to test the safety of different potential mesotherapy solutions in animals are planned in preparation for future human studies. Since we have shown that topical cream application will cause fat loss locally for cosmetic purposes, our next goal is to demonstrate that fat can be shifted from the hip and thighs to the breasts. In preparation for these studies, we have validated a cost-effective method of breast measurement. Thus, the studies in this thesis set the stage for a new chapter in cosmetic therapy, creams that will allow body sculpturing with fat augmentation and fat loss in different body areas. Not only does this offer the potential for improvements in quality of life, but it may also reduce the need for more risky and invasive plastic surgical procedures, thereby producing a public health benefit as well.

REFERENCES

Allison DB, Fontaine KR, Manson JE, et al: Annual deaths attributable to obesity in the United States. *JAMA* 1999; 282:1530-1538.

Anderson JW, Greenway FL, Fujioka K, Gadde KM, McKenney J, O'Neil PM. Bupropion SR enhances weight loss: a 48-week double-blind, placebo- controlled trial. *Obes Res* 2002 Jul;10(7):633-41.

Arner P, Arner O, Ostman J. The effect of local anesthetic agents on lipolysis by human adipose tissue. *Life Sci* 1973 Jul 16;13(2):161-9.

Astrup A, Breum L, Toubro S, Hein P, Quaade F. The effect and safety of an ephedrine/caffeine compound compared to ephedrine, caffeine and placebo in obese subjects on an energy restricted diet. A double blind trial. *Int J Obes Relat Metab Disord* 1992 Apr;16(4):269-77.

Bray GA. Coherent, preventive and management strategies for obesity. In: Chadwick DJ, Cardew G, eds. *The Origins and Consequences of Obesity* West Sussex, John Wiley, 1996, 228-246.

Brylinsky JA, Moore JC. The identification of body build stereotypes in young children. *J Res Pers* 1994;28:170-181.

Bulstrode N, Bellamy E, Shrotria S. Breast volume assessment: comparing five different techniques. *Breast* 2001;10:117-23.

Campaigne BN, Katch VL, Freedson P, Sady S, Katch FI. Measurement of breast volume in females: description of a reliable method. *Ann Hum Biol* 1979;6:363-7.

Caruso MK, Greenway FL, Hoffpauer DW. A rice bran bar containing omega-3 fatty acids and vitamins reduces serum homocysteine. *FASEB* 2005;19(5):A1010.

Caruso MK, Guillot TS, Nguyen T, Greenway FL. The cost effectiveness of three different measures of breast volume. *Aesthetic Plast Surg.* 2006 Jan-Feb;30(1):16-20.

Crandall CS. Do parents discriminate against their heavyweight daughters? *PSPB* 1995;21:724-735.

D'Costa MA, Asico W, Angel A. Inhibition of rat and human adipocyte adenylate cyclase in the antilipolytic action of insulin, clofibrate, and nicotinic acid. *Can J Biochem* 1979 Aug;57(8):1058-63.

Flegal KM. The obesity epidemic in children and adults: current evidence and research issues. *Med Sci Sports Exerc* 1999 Nov;31(11 Suppl):S509-14.

Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. *JAMA.* 2005 Apr 20;293(15):1861-7.

Freedman DS, Khan LK, Serdula MK, Galuska DA, Dietz WH. Trends and correlates of class 3 obesity in the United States from 1990 through 2000. *JAMA* 2002 Oct 9; 288(14):1772-1773.

Green A, Milligan G, Belt SE. Effects of prolonged treatment of adipocytes with PGE_1, N^6-phenylisopropyl adenosine and nicotinic acid on G-protein and anti-lipolytic sensitivity. *Biochemical Society Transactions* 19:212S, 1991.

Greenway FL. Surgery for obesity. *Endocrinol Metab Clin North Am* 1996; 25:1005-1027.

Greenway FL, Bray GA. Regional fat loss from the thigh in obese women after adrenergic modulation. *Clin Ther* 1987;9(6):663-9.

Greenway FL, Bray GA, Heber D. Topical Fat Reduction. *Obes Res* 1995 November; Suppl 4:561S-568S.

Greenway FL, Caruso MK. Safety of obesity drugs. *Expert Opin Drug Saf* 2005 Nov;4(6):1083-95.

Grossman AJ, Roudner LA. A simple means for accurate breast volume determination. *Plast Reconstr Surg* 1980;66:851-2.

Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *N Engl J Med* 2000 Dec 21;343(25):1833-8.

Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *JAMA* 2004 Jun 16;291(23):2847-50.

Komabayashi T, Sakamoto S, Tsuboi M. Effects of various drugs on the lipolytic actions caused by catecholamines and methylxanthine derivatives in white adipose tissues. 1. Effects of procaine and xylocaine. *Nippon Yakurigaku Zasshi* 1978 May;74(4):459-66.

Lafontan M, Dang-Tran L, Berlan M. Alpha-adrenergic antilipolytic effect of adrenaline in human fat cells of the thigh: comparison with adrenaline responsiveness of different fat deposits. *Eur J Clin Invest* 1979 Aug;9(4):261-6.

Large V, Peroni O, Letexier D, Ray H, Beylot M. Metabolism of lipids in human white adipocyte. *Diabetes Metab* 2004 Sep;30(4):294-309.

Latner JD, Stunkard AJ. Getting worse: the stigmatization of obese children. *Obes Res* 2003;11:452-456.

Loughry CW, Sheffer DB, Price TE, Jr., Lackney MJ, Bartfai RG, Morek WM. Breast volume measurement of 248 women using biostereometric analysis. *Plast Reconstr Surg* 1987;80:553-8.

Mersmann HJ. Effect of anesthetic or analgesic drugs on lipogenic and lipolytic adipose tissue activities. *Proc Soc Exp Biol Med* 1983 Mar;172(3):375-8.

Niaspan® Package Insert. Kos Pharmaceuticals, Inc., Miami, Florida 33131, USA, 2003.

Obesity: Preventing and Managing the Global Epidemic. *Report of a World Health Organization Consultation on Obesity*, Geneva, 1997 June 3-5;208-209.

Palin WE, Jr., von Fraunhofer JA, Smith DJ, Jr. Measurement of breast volume: comparison of techniques. *Plast Reconstr Surg* 1986;77:253-5.

Pechter EA. A new method for determining bra size and predicting postaugmentation breast size. *Plast Reconstr Surg* 1998;102:1259-65.

Peiris AN, Hennes MI, Evans DJ, Wilson CR, Lee MB, Kissebah AH. Relationship of anthropometric measurements of body fat distribution to metabolic profile in premenopausal women. *Acta Med Scand Suppl* 1988;723:179-88.

Peiris AN, Mueller RA, Struve MF, Smith GA, Kissebah AH. Relationship of androgenic activity to splanchnic insulin metabolism and peripheral glucose utilization in premenopausal women. *J Clin Endocrinol Metab* 1987 Jan;64(1):162-9.

Pierard GE, Nizet JL, Pierand-Franchimont C. Cellulite: From standing fat herination to hypodermal stretch marks. *Am J Dermatopathol* 2000 Feb;22(1):34-7.

Pistor M. Mesotherapy. *Librairie Maloine*, Paris, 1964.

Puhl R, Brownell KD. Bias, discrimination, and obesity. *Obes Res* 2001;9:788-805.

Putnam JJ. Cases of myxoedema and acromegalia treated with benefit by sheep's thyroids. Am *J Med Sci* 1893;106(2):125-148.

Querleux, B, Cornillon C, Jolivet O, Bittoun J. Anatomy and physiology of subcutaneous adipose tissue by in vivo magnetic resonance imaging and spectroscopy: relationships with sex and presence of cellulite. *Skin Res Technol* 8, 118-124, 2002.

Roberts AT, Martin CK, Liu Z, Amen RJ, Woltering EA, Rood JC, Caruso MK, Yu Y, Xie H, Greenway FL, 2005. The safety and efficacy of a dietary herbal supplement and gallic acid for weight loss. Submitted to *Int J Obes,* 2005.

Roberts AT, Caruso MK, Yu Y, Woltering EA, Liu Z, Schwimer JE, Bellanger DE, Guillot TS, Greenway FL. Validation of an angiogenesis assay based on human fat tissue. Presented at NAASO Annual Scientific Meetings, Vancouver, British Columbia, October 15-19, 2005, and submitted to *Obes Res,* 2005.

Robidoux J, Martin TL, Collins S. Beta-adrenergic receptors and regulation of energy expenditure: a family affair. *Annu Rev Pharmacol Toxicol* 2004;44:297-323.

Sironi AM, Gastaldelli A, Mari A, Ciociaro D, Postano V, Buzzigoli E, Ghione S, Turchi S, Lombardi M, Ferrannini E. Visceral fat in hypertension: influence on insulin resistance and beta-cell function. *Hypertension* 2004 Aug;44(2):127-33. Epub 2004 Jul 19.

Soutter WP, Hanoch J, D'Arcy T, Dina R, McIndoe GA, DeSouza NM. Pretreatment tumour volume measurement on high-resolution magnetic resonance imaging as a predictor of survival in cervical cancer. *Bjog* 2004;111:741-7.

Stevens J, Cai J, Pamuk ER, Williamson DF, Thun MJ, Wood JL. The effect of age on the association between body-mass index and mortality. *N Engl J Med* 1998; 338:1-7.

Tezel E, Numanoglu A. Practical do-it-yourself device for accurate volume measurement of breast. *Plast Reconstr Surg* 2000;105:1019-23.

Tunaru S, Kero J, Schaub A, Wufka C, Blaukat A, Pfeffer K, Offermanns S. PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. *Nat Med* 2003 Mar;9(3):352-5. Epub 2003 Feb 03.

Vukovich MD, Schoorman R, Heilman C, Jacob P 3rd, Benowitz NL. Caffeine-herbal ephedra combination increases resting energy expenditure, heart rate and blood pressure. *Clin Exp Pharmacol Physiol* 2005 Jan-Feb;32(1-2):47-53.

Wolf AM, Colditz GA. Current estimates of the economic cost of obesity in the United States. *Obes Res* 1998 Mar;6(2):97-106.

APPENDIX A: COPYRIGHT PERMISSION FOR PUBLISHED ARTICLE

From: Mary Caruso
To: permissions.heidelberg@springer.com
Date: 3/13/2006 3:21:31 PM
Subject: permission to use published material

Hi,

My article was published in the Jan-Feb issue of Aesthetic Plastic Surgery, Springer publishers. I am the first author of the article and am using it in my thesis. I need written permission to use my article. My information that you requested is as follows:

Mary Katherine Caruso
Pennington Biomedical Research Center
6400 Perkins Rd.
Baton Rouge, LA 70808
Fax:225-763-3022
Phone:225-763-2699
carusomk@pbrc.edu

Please let me know if you need anything else.

Here is the following information about my article:

Caruso MK, Guillot TS, Nguyen T, Greenway FL.
The cost effectiveness of three different measures of breast volume.
Aesthetic Plast Surg. 2006 Jan-Feb;30(1):16-20.
PMID: 16411159 [PubMed - in process]

Sincerely,
Mary Katherine

APPENDIX B: COPYRIGHT PERMISSION FOR ACCEPTED ARTICLE

From: "Journals Rights" <JournalsRights@oxon.blackwellpublishing.com>
To: "Mary Caruso" <CarusoMK@pbrc.edu>
Date: 3/20/2006 3:14:42 AM
Subject: RE: copyright permission request

Thank you for your email request. Permission is granted for you to use the material below for your thesis subject to the usual acknowledgements and on the understanding that you will reapply for permission if you wish to distribute or publish your thesis commercially.

Good Luck!

Permissions Dept.
Blackwell Publishing
PO Box 805
9600 Garsington Road
Oxford
OX4 2ZG
United Kingdom
Fax: 00 44 1865 471150

Permission requests can now be sent to journalsrights@oxon.blackwellpublishing.com

Blackwell is committed to creating a culture of value and respect for all of our staff. We expect to work in an environment where there are high standards of behaviour and achievement. We maintain a culture which operates within accepted boundaries of professional behaviour and performance.

-----Original Message-----
From: Mary Caruso [mailto:CarusoMK@pbrc.edu]
Posted At: 17 March 2006 21:21
Posted To: March 2006
Conversation: copyright permission request
Subject: copyright permission request

Hi,

My article titled "Topical Fat Reduction from the Waist" has recently been accepted to Diabetes, Obesity and Metabolism. I am the first author of the article, and need written permission to use the article in my thesis. Could you please provide me with a letter? All I need is a quick email saying that I have received permission to use the article in my thesis. Here is my information:

Mary Katherine Caruso
Pennington Biomedical Research Center
6400 Perkins Rd.
Baton Rouge, LA 70808
phone: 225-763-2699
fax: 225-763-3022

Please let me know if you need anything else!

Thank you,
Mary Katherine Caruso

VITA

Mary Katherine Caruso was born on May 15, 1981, to parents Luke and Dana Caruso in Metairie, Louisiana. She is the middle child to her older sister Angela and younger brother Tony. Katie, as called by everyone, graduated from Mount Carmel Academy in May of 1999. That fall, she went on to attend Louisiana State University and graduated in the fall of 2003 with a Bachelor of Science degree in biological sciences. Katie then entered a graduate program in nutrition in the fall of 2004. Over the past four years, she has been working at Pennington Biomedical Research Center, and she has been a research assistant and study coordinator there for the past two years under the direction of Dr. Frank Greenway.

Katie was a presenter at the annual 2005 Experimental Biology conference in San Diego. Her poster was titled "A Rice Bran Bar Containing Omega-3 Fatty Acids and Vitamins Reduces Serum Homocysteine", and the article appeared in the March 2005 issue of *The FASEB Journal.* She was also a presenter at the annual 2005 North American Association for the Study of Obesity conference in Vancouver. Her poster was titled "An Evaluation of Solutions Used in Mesotherapy for Lipolysis and Body Recontouring". Katie is the co-author of "Safety of Obesity Drugs" which appeared in the November 2005 issue of *The Expert Opinion on Drug Safety.* She is also the author of "The Cost Effectiveness of Three Different Measures of Breast Volume" which appeared in the January 2006 issue of *Aesthetic Plastic Surgery.* She also has another article that has been accepted and three articles that have been submitted to peer-reviewed scientific journals. Katie will graduate in the spring of 2006 with a Master of Science degree in human nutrition and foods and has plans to marry her fiancé Eric in June of 2007.